Changes in the Lymphocyte Cytoplasmic Refractive Index Following Typhoid Vaccination

J. G. WILDE* AND W. K. METCALF, M.D.

*University of Iowa Medical School,
Iowa City, IA 52240
and
University of Nebraska Medical Center,
Omaha, NB 68105

ABSTRACT

The refractive index of the circulating blood lymphocytes (LCRI) of healthy young adults vaccinated with typhoid vaccine has been measured by immersion refractometry and phase contrast microscopy. A rise in the LCRI precedes the rise in the antibody titre by two to three days. A more pronounced rise also occurred in one patient prior to a rejection crises following a kidney transplant.

The refractive index of the cytoplasm of circulating blood lymphocytes (LCRI) can readily be measured by phase contrast microscopy and immersion refractometry. It is probably a function of the cytoplasmic protein concentration. This parameter has been shown to rise under conditions when an immune response might be expected, e.g., following typhoid vaccination in rats, following skin autografting but not homografting in rabbits, in human pregnancy and in patients with malignant tumors. The purpose of this work was to determine whether or not the LCRI of humans responds in the same way as that of rats to typhoid vaccination and to relate the timing of any change to that of the change in the antibody titer.

Materials and Methods

The technique has been described in detail previously. Briefly, 10 ml of heparinized blood is collected by venipuncture using a vacutainer. The blood sample may, if necessary, be stored overnight at 4°. Lymphocytes are separated by the Kaplow buffy coat method. Aliquots of the lymphocyte suspension are mixed with solutions of isotonic bovine serum albumin (BSA) that differ by small increments of refractive index. A slide is prepared for each dilution of BSA, and the lymphocytes on each slide are examined with a 40× phase contrast microscope.

If the lymphocyte cytoplasm has a refractive index greater than the background BSA, the cytoplasm will appear darker than the background medium. If the cytoplasm's refractive index is less than the background solution's, the cytoplasm will appear lighter. When the shading of the cytoplasm matches the background BSA, the cytoplasm and the BSA have the same refractive index. For a given blood sample,
the refractive index of the lymphocyte’s cytoplasm is determined by counting approximately 20 cells per slide and by indicating the number of cells that have cytoplasm darker, lighter or equal in shade to the background BSA. The refractive index of the suspension, from which the slide which had the majority of its lymphocytes with cytoplasm equal in shade to that of the background BSA and approximately equal numbers of lymphocytes with cytoplasm lighter and darker than the background, is measured using a temperature compensated refractometer. This value is taken as the refractive index of the lymphocyte’s cytoplasm for that sample of blood. Antibody titers were determined, when appropriate, by the plate agglutination method using a commercial S. typhosa H (flagellar) antigen.

**Results**

A normal mean LCRI value of 1.3562 ± 0.0006 was determined by measuring 30 samples from 17 people of both sexes. This is equivalent to a protein concentration of 11.4 g per dl ± 0.3 g per dl. The range for these 30 samples was from 10.5 g per dl to 11.8 g per dl of protein. Six tests were done on one individual over a period of three months. This yielded a value of 11.6 g per dl of protein ± 0.15 g per dl with a range of 11.4 g per dl to 11.7 g per dl. These data seem to indicate that although slight individual variations do exist, the value is a stable one.

Following the determination of a normal LCRI value, six volunteers received typhoid vaccinations. Blood samples were drawn intermittently for 14 to 16 days following vaccination. The results (figure 1) show an increase in the LCRI, beginning about six days following vaccination. The rise in antibody titer followed the rise in LCRI two to four days later (figure 2).

An anamnestic response was studied in one individual who had been vaccinated against typhoid two years earlier. Although no residual antibody titer was detected, the LCRI and antibody titer increases were
both more pronounced. The increase represented a change of protein concentration of 2.7 g per dl by day six as compared to an increase of 0.6 g per dl of protein by day eight in the group of primary responses.

The LCRI was also monitored in a patient who had a kidney transplanted from a cadaver donor. The LCRI began to increase by the 10th post-operative day, and on the 15th post-operative day a biopsy showed a rejection to be occurring. At the time of biopsy, the LCRI had risen the equivalent of 2.4 g per dl of protein, and an increase of 1.1 g per dl of protein had occurred approximately five days before the rejection crisis was detected (figure 3). Following the biopsy, the patient’s immunosuppressive therapy was increased so that it became impossible to isolate enough lymphocytes to make further determinations. However, two weeks later, when the dosage of immunosuppressives had been decreased, the patient’s LCRI had returned to normal.

Discussion

It would seem that the LCRI is an indicator of some type of lymphocyte response to an antigenic challenge. A rise in the LCRI is elicited both by a vaccine that confers an immunity that is in part cell mediated and by a tissue rejection phenomenon that is also cell mediated. Therefore, it would not seem unreasonable to interpret a rise in the LCRI following an antigenic challenge as a lymphocyte mediated response to that challenge manifested by an increase in protein synthesis which results in an increase in the protein concentration of the lymphocyte cytoplasm. This increase in the LCRI apparently involves at least 75 percent of the circulating lymphocytes, which is a percentage roughly equivalent to the T cell population of peripheral blood as measured by PHA* responsiveness. One interpretation of this phenomenon is that it is, in some way, correlated with antigen processing rather than antibody synthesis, a conclusion supported by the return to normal of the LCRI at a time when the antibody titer is still rising. An increase in LCRI which almost certainly indicates a rise in cytoplasmic protein concentration, apparently also occurs in pregnancy following skin autografting, in patients with malignant disease and in patients with rheumatoid arthritis, probably an autoimmune phenomenon. The recent report of Lawton and Kelton that lymphocyte specific gravity distributions show marked differences in a similar set of circumstances is of interest in that specific gravity is likely to be related to refractive index. However, insufficient data are presented to allow detailed correlations. Both techniques show promise for clinical application and clearly merit further investigation.

* Phytohemagglutinin.
References